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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/513,362
Filing Date: February 25, 2000
Appellant(s): CHEE ET AL.

Astrid R. Spain
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed December 14, 2007 appealing from the Office action mailed November 30, 2007

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

No amendment after final has been filed.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

6,274,320 B1	Rothberg et al.	8-2001
6,327,410 B1	Walt et al.	12-2001
WO 98/13523	Nyren et al.	4-1998

WO 91/06678 Ross et al. 5-1991

Stratagene Catalog, "Gene Characterization Kits", p. 39 (1988)

Michael, K. L. et al., "Randomly Ordered Addressable High-Density Optical Sensor Arrays", Anal. Chemistry, vol. 70, pp. 1242-1248 (1998)

Ronaghi, M. et al. "Real-time DNA Sequencing Using Detection of Pyrophosphate Release", Anal. Biochemistry, vol. 242, pp. 84-89 (1996)

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Interpretation

1. The term "covalent attachment" has been defined by Applicants on page 27, lines 9-11 in the following way:

"By "covalently attached" herein is meant that two moieties are attached by at least one bond, including sigma bonds, pi bonds and coordination bonds."

However, Applicants also use the term in a broader sense, by describing covalent immobilization of hybridization complexes onto the support, where only one of the elements of the hybridization probe, namely, the capture probe, is covalently attached to the support (page 3, lines 14-16 and 27-30):

"The hybridization complexes comprise the target sequence, the sequencing primer and a capture probe covalently attached to the surface."

"The method comprises providing a hybridization complex comprising the target sequence and a capture probe covalently attached to microspheres on a surface of a substrate and determining the identity of a plurality of bases at the target positions. The hybridization complex

comprises the capture probe, an adapter probe, and the target sequence. In one aspect the sequencing primer is the capture probe."

Therefore, the term "covalent attachment" as applied to more than one element is therefore interpreted as meaning that at least one element of the hybridization complex is covalently attached to the support.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 1-16, 22-27, 31-38, 40-42, 44-47, 49 and 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rothberg et al. (U.S. Patent No. 6,274,320 B1; cited in the IDS and in the previous office action) and Walt et al. (U.S. Patent No. 6,327,410 B1; cited in the previous office action).

Claims 1, 10 and 34 will be considered together, since claim 1 is a species of claim 34 and differs from claim 10 by a limitation of sequencing primers covalently attached to the microspheres.

Regarding claims 1, 10 and 34 Rothberg et al. teach a method of sequencing nucleic acids, the method comprising:

a) providing an array comprising:

i) a substrate with a surface comprising discrete sites (Rothberg et al. teach a substrate, which is a fiber optic surfaces, comprising discrete sites (col. 2, lines 66, 67; col. 3, lines 1-6).); and

iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate (Rothberg et al. teach enzymes which generate signal from pyrophosphate attached at discrete sites on the substrate (col. 4, lines 16-28; col. 18, lines 4-8).);

b) providing a first hybridization complex comprising said first domain of a first target sequence and a first sequence primer, wherein said first hybridization complex is attached to said first subpopulation (Rothberg et al. teach providing a first hybridization complex comprising a first domain of a first target sequence and a first sequence primer, where the first hybridization complex is attached to the surface of the support (Fig. 1; col. 3, lines 18-30).);

c) providing second hybridization complex comprising said second domain of a second target sequence and a second sequence primer, wherein said second hybridization complex is attached to said second subpopulation (Rothberg et al. teach providing at least one hybridization complex by providing a plurality of anchor primers and a plurality of nucleic acid templates (= targets) (col. 3, lines 18-30 and 41-45; col. 5, lines 6-15). Rothberg et al. also teach libraries of nucleic acid templates, therefore they inherently teach templates with different sequences (col. 9, lines 25-28; col. 10, lines 32-58).);

d) simultaneously extending said first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively (Rothberg et al. teach simultaneous addition of a first nucleotide to the first

and second hybridization complexes by a polymerase to generate extended primers (col. 3, lines 31-33; col. 14, lines 15-21).);

e) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete site within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide added onto said first and second primers, respectively (Rothberg et al. teach detection of the pyrophosphate by the enzymes immobilized on the substrate within a common reaction chamber (col. 4, lines 11-28; col. 14, lines 35-41 and 64-66; col. 16, lines 7-14; col. 17, lines 1-9 and 63-67; col. 18, lines 1-8 and 47-67; col. 19, lines 1-8; Fig. 2 and 3; col. 27, lines 58-67; col. 28, lines 1-12 and 60-65).); and

f) determining sequences for said plurality of target nucleic acids (Rothberg et al. teach determining the sequences of the nucleic acids (col. 3, lines 33-40; col. 14, lines 50-56; col. 17, lines 24-36).).

Regarding claim 2, Rothberg et al. teach covalent attachment of anchor primers to the support (col. 7, lines 41, 42) and formation of the hybridization complex on the anchor primer (Fig. 1; col. 3, lines 18-30), therefore they teach covalent attachment of hybridization complexes to the support.

Regarding claim 3, Rothberg et al. teach covalent attachment of anchor primers to the support (col. 7, lines 41, 42) and formation of the hybridization complex on the anchor primer (Fig. 1; col. 3, lines 18-30), therefore they teach attachment of sequencing primers to the support.

Regarding claims 4 and 11, Rothberg et al. teach covalent attachment of anchor primers to the support (col. 7, lines 41, 42) and formation of the hybridization complex on the anchor primer (Fig. 1; col. 3, lines 18-30). Since the anchor primers are capture probes (Fig. 1),

Rothberg et al. teach hybridization complexes comprising capture probes and covalent attachment of capture probes to the support.

Regarding claims 5 and 33, Rothberg et al. teach covalent attachment of anchor primers to the support (col. 7, lines 41, 42) and formation of the hybridization complex on the anchor primer (Fig. 1; col. 3, lines 18-30). Since the anchor primers are capture probes which comprise adapter sequences (Fig. 1), Rothberg et al. teach hybridization complexes comprising capture probes and adapter sequences and covalent attachment of hybridization complexes to the support.

Regarding claim 6, Rothberg et al. teach addition of a second nucleotide to the targets using polymerase and detecting the pyrophosphate to determine the second nucleotide (col. 3, lines 37-40; col. 17, lines 24-36).

Regarding claims 7-9, Rothberg et al. teach detecting the PPi by contacting the PPi with ATP sulfurylase (= second enzyme) that converts PPi to ATP and detecting the ATP using a luciferase (= third enzyme) which generates light, the enzymes being attached to the solid support (col. 4, lines 16-29; col. 14, lines 64-66; col. 16, lines 7-20 and 32-34; col. 18, lines 4-8).

Regarding claims 10 and 12, Rothberg et al. teach covalent immobilization of primers (col. 7, lines 41, 42). Further, since the sequencing primers are a part of the hybridization complex which is covalently attached to the support (Fi. 1), they are covalently attached in that sense as well.

Regarding claim 13, Rothberg et al. teach:

a) providing a sequencing primer hybridized to said second domain (Rothberg et al. teach providing a sequencing primer which anneals to the target domain (Fig. 1; col. 3, lines 27-30).);

b) extending said primer by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer (Rothberg et al. teach addition of a first nucleotide to the first detection position by a polymerase to generate extended primers (col. 3, lines 31-33; col. 14, lines 15-21).);

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer (Rothberg et al. teach detection of the pyrophosphate to determine the type of the nucleotide added to the primer (col. 3, lines 30-37; col. 14, lines 35-41).);

d) extending said primer by the addition of a second nucleotide to a second detection position using said enzyme (Rothberg et al. teach addition of a second nucleotide to the second detection position (col. 3, lines 30-37; col. 14, lines 13-21).); and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer (Rothberg et al. teach detection of the pyrophosphate to determine the type of the nucleotide added to the primer (col. 3, lines 30-37; col. 14, lines 35-41).).

Regarding claims 14-16, Rothberg et al. teach detecting the PPi by contacting the PPi with ATP sulfurylase (= second enzyme) that converts PPi to ATP and detecting the ATP using a luciferase (= third enzyme) which generates light, the enzymes being attached to the solid support (col. 4, lines 16-29; col. 14, lines 64-66; col. 16, lines 7-20 and 32-34; col. 18, lines 4-8).

Regarding claims 22-25, Rothberg et al. teach discrete sites on the fiber optic being wells (Fig. 4; col. 6, lines 64-66; col. 7, lines 1-10; col. 20, lines14-24).

Regarding claim 26, Rothberg et al. teach a substrate comprising a fiber optic bundle (col. 2, lines 66, 67; col. 3, lines 1-6; Fig. 2).

Regarding claim 27, Rothberg et al. teach glass and plastic supports (col. 2, lines 18-20; col. 19, line 41).

Regarding claims 34, 37 and 42, Rothberg et al. teach genomic DNA (col. 34-38).

Regarding claims 35 and 38, Rothberg et al. teach enzymes attached to the substrate (col. 4, lines 26-28).

Regarding claims 36 and 41, Rothberg et al. teach PCR products as targets (col. 11, lines 9-30).

Regarding claim 40, Rothberg et al. teach target sequences covalently attached to the substrate (col. 14, lines 12-15; col. 7, lines 41, 42).

Regarding claims 44, 46 and 49, Rothberg et al. teach a flow cell (Fig. 3; col. 4, lines 66, 67).

Regarding claims 45, 47 and 50, Rothberg et al. teach washing away unreacted nucleotides (col. 17, lines 14-19).

B) Rothberg et al. teach attachment of reactants to the surface of the fiber optic, but do not teach microspheres on the surface of the fiber optic bundle.

C) Regarding claims 1, 10 and 34, Walt et al. teach microsphere-based analytical chemistry system in which the microspheres are distributed on a fiber optic bundle (Abstract). The surface of the substrate comprises discrete sites into which at least two subpopulations of microspheres are distributed. Each of the microspheres comprises a bioactive agent and an optical signature which allows identification of the bioactive agent. The beads are randomly

distributed on the array (col. 3, lines 35-45; col. 4, lines 54-56). The bioactive agent attached to the microsphere is a nucleic acid, particularly a nucleic acid probe (col. 7, lines 55-66; col. 8, lines 15-19; col. 9, lines 41-67; col. 10, lines 1-47). The array is used for sequencing (col. 24, lines 51-52).

Regarding claims 22-25, Walt et al. teach substrate with discrete sites, first and second poupulations of the microspheres (col. 3, lines 35-40), the discrete sites being wells (Fig. 5; col. 6, lines 22-24) and microspheres randomly distributed in these sites (col. 4, lines 54-56). Walt et al. teach the substrate being a fiber optic bundle (col. 6, lines 32-35).

Regarding claim 31, Walt et al. teach decoding of the array prior to the testing performed on the array (col. 4, lines 56-58; col. 22, lines 19-25).

Regarding claim 32, Walt et al. teach the microspheres containing a probe (=identifier binding ligand) which binds a decoder binding ligand (= target nucleic acid) (col. 10, lines 43-47; col. 21, lines 17-60). Since each of the beads contains a unique optical signature (col. 13, lines 8-24), the identity and location of each bead can be determined.

Regarding claims 35 and 38, Walt et al. teach enzymes immobilized on microspheres (col. 20, lines 51-67; col. 25, lines 57-67; col. 26, lines 1-25).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the microspheres of Walt et al. distributed over the surface of the fiber optic sensor in the method of nucleic acid sequencing of Rothberg et al. The motivation to do so, provided by Walt et al., would have been that (col. 3, lines 13-26):

> "The innovation of the two previous patents was the placement of multiple chemical functionalities at the end of a single optical fiber bundle sensor. This configuration yielded an analytic chemistry sensor that could be remotely monitored via the typically small bundle.

The drawback, however, was the difficulty in applying the various chemistries associated with the chemical functionalities at the sensor's end; the functionalities were built on the sensor's end in a serial fashion. This was a slow process, and in practice, only tens of functionalities could be applied. Accordingly, compositions and methods are desirable that allow the generation of large fiber optic arrays including microspheres that can be either encoded or decoded to allow the detection of target analytes."

and (col. 4, lines 35-56):

"The present invention is based on two synergistic inventions: 1) the development of a bead-based analytic chemistry system in which beads, also termed microspheres, carrying different chemical functionalities may be mixed together while the ability is retained to identify the functionality of each bead using an optically interrogatable encoding scheme (an "optical signature"); and 2) the use of a substrate comprising a patterned surface containing individual sites that can bind or associate individual beads. This allows the synthesis of the bioactive agents (i.e. compounds such as nucleic acids and antibodies) to be separated from their placement on an array, i.e. the bioactive agents may be synthesized on the beads, and then the beads are randomly distributed on a patterned surface. Since the beads are first coded with an optical signature, this means that the array can later be "decoded", i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the in situ synthesis or spotting techniques of the prior art."

4. Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rothberg et al. (U.S. Patent No. 6,274,320 B1; cited in the IDS and in the previous office action) and Walt et al. (U.S. Patent No. 6,327,410 B1; cited in the previous office action), as applied to claim 10 above, and further in view of Nyren et al. (WO 98/13523; cited in the previous office action).

A) Rothberg et al. teach pyrosequencing using nucleotides, but do not teach protected nucleotides.

B) Nyren et al. teach pyrosequencing (Abstract) using 3'protected nucleotides (page 17, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the 3'-protected nucleotides of Nyren et al. in the method of pyrosequencing of Rothberg et al. and Walt et al. The motivation to do so, provided by Nyren et al., would have been that using protected nucleotides allowed chain extension to proceed one position at a time without complications caused by sequences of identical bases (page 17, third paragraph).

5. Claims 18, 19, 28-30, 43 and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rothberg et al. (U.S. Patent No. 6,274,320 B1; cited in the IDS and in the previous office action), Walt et al. (U.S. Patent No. 6,327,410 B1; cited in the previous office action), Nyren et al. (WO 98/13523; cited in the previous office action) and Stratagene Catalog (1988, p. 39; cited in the previous office action).

A) Regarding claim 18, Rothberg et al. teach a kit comprising:

a) composition comprising:

i) a substrate with a surface comprising discrete sites (Rothberg et al. teach a surface of a substrate comprising discrete sites, for example, pads with attached anchor primers or wells (col. 2, lines 66, 67; col. 3, lines 1-6; col. 6, lines 64-67; col. 7, lines 1-15; col. 20, lines 15-18).);

ii) a population of microspheres distributed on said sites, wherein said

microspheres comprise different capture probes, wherein said array is configured for simultaneous contact of said different capture probes with a common reaction chamber (Rothberg et al. teach a substrate with a plurality of anchor primers (=capture probes) (Fig. 1; col. 3, lines 19-24 and 42-45). Rothberg et al. teach simultaneous contact of different capture probes in a common reaction chamber (col. 17, lines 63-67; col. 18, lines 1-3 and 40-67; Fig. 2).); and

iii) an enzyme attached at said discrete sites wherein said enzyme is used to generate a signal from pyrophosphate (Rothberg et al. teach enzymes ATP sulfurylase and luciferase immobilized on solid support and generation of signal from pyrophosphate using these enzymes (col. 3, lines 11-22; col. 18, lines 4-8).);

b) a first extension enzyme (Rothberg et al. teach extension of the sequencing primer with a polymerase (= first enzyme) (col. 3, lines 30-33; col. 22, lines 45-55).); and

c) dNTPS (Rothberg et al. teach dNTPs (col. 17, lines 24-30).).

Regarding claim 19, Rothberg et al. teach detection of pyrophosphate using ATP sulfurylase (= second enzyme) (col. 14, lines 64-66) and detection of ATP using luciferase (= third enzyme) (col. 16, lines 7-20 and 33-35), with the enzymes attached to solid support (col. 3, lines 11-22; col. 18, lines 4-8).

Regarding claim 28, Rothberg et al. teach wells on the surface of the fiber optic bundle (Fig. 4; col. 6, lines 64-66; col. 7, lines 1-10; col. 20, lines14-24).

Regarding claim 29, Rothberg et al. teach a substrate comprising a fiber optic bundle (col. 2, lines 66, 67; col. 3, lines 1-6; Fig. 2).

Regarding claim 30, Rothberg et al. teach glass and plastic supports (col. 2, lines 18-20; col. 19, line 41).

Regarding claim 43, Rothberg et al. teach enzymes attached to the substrate (col. 4, lines 26-28).

Regarding claim 48, Rothberg et al. teach a flow cell (Fig. 3; col. 4, lines 66, 67).

B) Rothberg et al. teach attachment of reactants to the surface of the fiber optic, but do not teach microspheres on the surface of the fiber optic bundle.

C) Regarding claim 18, Walt et al. teach microsphere-based analytical chemistry system in which the microspheres are distributed on a fiber optic bundle (Abstract). The surface of the substrate comprises discrete sites into which at least two subpopulations of microspheres are distributed. Each of the microspheres comprises a bioactive agent and an optical signature which allows identification of the bioactive agent. The beads are randomly distributed on the array (col. 3, lines 35-45; col. 4, lines 54-56). The bioactive agent attached to the microsphere is a nucleic acid, particularly a nucleic acid probe (col. 7, lines 55-66; col. 8, lines 15-19; col. 9, lines 41-67; col. 10, lines 1-47). The array is used for sequencing (col. 24, lines 51-52).

Regarding claim 43, Walt et al. teach enzymes immobilized on microspheres (col. 20, lines 51-67; col. 25, lines 57-67; col. 26, lines 1-25).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the microspheres of Walt et al. distributed over the surface of the fiber optic sensor in the method of nucleic acid sequencing of Rothberg et al. The motivation to do so, provided by Walt et al., would have been that (col. 3, lines 13-26):

"The innovation of the two previous patents was the placement of multiple chemical functionalities at the end of a single optical fiber bundle sensor. This configuration yielded an analytic chemistry sensor that could be remotely monitored via the typically small bundle. The drawback, however, was the difficulty in applying the various chemistries associated with the chemical functionalities at the sensor's end; the functionalities were built on the sensor's end in a serial fashion. This was a slow process, and in practice, only tens of functionalities could be applied. Accordingly, compositions and methods are desirable that allow the generation of large fiber optic arrays including microspheres that can be either encoded or decoded to allow the detection of target analytes."

and (col. 4, lines 35-56):

"The present invention is based on two synergistic inventions: 1) the development of a bead-based analytic chemistry system in which beads, also termed microspheres, carrying different chemical functionalities may be mixed together while the ability is retained to identify the functionality of each bead using an optically interrogatable encoding scheme (an "optical signature"); and 2) the use of a substrate comprising a patterned surface containing individual sites that can bind or associate individual beads. This allows the synthesis of the bioactive agents (i.e. compounds such as nucleic acids and antibodies) to be separated from their placement on an array, i.e. the bioactive agents may be synthesized on the beads, and then the beads are randomly distributed on a patterned surface. Since the beads are first coded with an optical signature, this means that the array can later be "decoded", i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the in situ synthesis or spotting techniques of the prior art."

D) Neither Rothberg et al. nor Walt et al. teach kits.

E) Nyren et al. teach a kit for sequencing of DNA by pyrophosphate release, the kit comprising a sequencing primer, a polymerase, a detection enzyme means for identifying

pyrophosphate release, dNTPs or ddNTPs (page 20, second paragraph; page 21, first paragraph).

F) Stratagene catalog teaches a motivation to combine reagents into kit format (page 39).

It would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine the method of Rothberg et al. and Walt et al. into a kit format as discussed by Stratagene catalog and suggested by Nyren et al., since the Stratagene catalog teaches a motivation for combining reagents of use in an assay into a kit (page 39, column 1),

"Each kit provides two services: 1) a variety of different reagents have been assembled and pre-mixed specifically for a defined set of experiments. Thus one need not purchase gram quantities of 10 different reagents, each of which is needed in only microgram amounts, when beginning a series of experiments. When one considers all of the unused chemicals that typically accumulate in weighing rooms, desiccators, and freezers, one quickly realizes that it is actually far more expensive for a small number of users to prepare most buffer solutions from the basic reagents. Stratagene provides only the quantities you will actually need, premixed and tested. In actuality, the kit format saves money and resources for everyone by dramatically reducing waste. 2) The other service provided in a kit is quality control".

6. Claims 20 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rothberg et al. (U.S. Patent No. 6,274,320 B1; cited in the IDS cited in the previous office action), Walt et al. (U.S. Patent No. 6,327,410 B1; cited in the previous office action), Nyren et al. (WO 98/13523; cited in the previous office action) and Stratagene Catalog (1988, p. 39; cited in the previous office action), as applied to claim 18 above, and further in view of Ross et al. (WO 91/06678; cited in the previous office action).

A) Teachings of Rothberg et al., Walt et al., Nyren et al. and Stratagene Catalog are presented above. None of these references teaches labeled nucleotides or different labels on nucleotides.

B) Ross et al. teach sequencing of nucleic acids by sequential addition of 3'-blocked nucleotides to the template (Abstract; page 11, lines 28-36; page 12; page 13, lines 1-29). The different types of nucleotides are labeled with different labels (page 12, lines 14-18).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used labeled nucleotides of Ross et al. in the sequencing kit of Rothberg et al., Walt et al., Nyren et al. and Stratagene Catalog. The motivation to do so, provided by Ross et al., would have been that incorporation of nucleotides was monitored by detecting the label on the dNTP (page 26, lines 1-5) and using fluorescent labels increased detection sensitivity (page 31, lines 1-5).

(10) Response to Argument

Issues

I. Whether the statement of motivation to combine Rothberg et al. with Walt et al. has been provided in the office action (as applied to claims 1-16, 22-27, 31-38, 40-42, 44-47, 49 and 50)?

II. Whether there is motivation to combine Rothberg et al. with Walt et al. considering alleged teaching away from using beads by Rothberg et al. (as applied to claims 1-16, 22-27, 31-38, 40-42, 44-47, 49 and 50)?

III. Whether claim 17 is unpatentable over Rothberg et al., Walt et al. and Nyren et al.; whether claims 18, 19, 28-30, 43 and 48 are unpatentable over Rothberg et al., Walt et al., Nyren et al. and Stratagene Catalog; and whether claims 20 and 21 are unpatentable over Rothberg et

al., Walt et al., Nyren et al., Stratagene Catalog and Ross et al. considering Rothberg's teaching away from using beads and lack of motivation to combine Rothberg et al. with Walt et al.?

Regarding I, Appellant's argument was addressed in the previous office actions, but is reiterated here for convenience of the Board.

The motivation statement in paragraph 3 of the office action reads: "It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to <u>have used the microspheres of Walt et al. distributed over the surface of the fiber optic sensor in the method of nucleic acid sequencing of Rothberg et al</u>. The motivation to do so, provided by Walt et al., would have been that (col. 3, lines 13-26):.." (emphasis added). Therefore, contrary to Appellant's allegation, this element of the motivation statement is in fact present in the rejection. Further, the motivation statement included in the rejection specifically details the benefits one of ordinary skill would obtain by using the beads of Walt et al. distributed over a fiber optic surface rather than having components of the reaction immobilized directly to the fiber optic, as taught by Rothberg et al. As the statements of Walt et al. are rather self-explanatory, one of ordinary skill in the art would not need any further explanatory comments added to understand the benefits offered by the bead system.

Regarding II, there are several issues connected to the alleged statement of teaching away from using beads by Rothberg et al. They are as follows:

A) Do Rothberg et al. really "teach away" from using beads in their invention, i.e., would one of ordinary skill in the art be discouraged from using beads of Walt et al. in the method of Rothberg et al.?

B) Significance of bead loss for the performance of the method.

C) Appellant's solution to the problem of bead loss.

Let us start from Appellant's claimed invention: it is a method of performing sequencing of nucleic acids by detecting pyrophosphate (PPi) released by a polymerase during the sequencing reaction, where some of the reaction components are immobilized on beads distributed in discrete sites on a surface of a substrate. No details of the structure of the support or beads are claimed. Rothberg et al. teach pyrosequencing performed on a surface of a fiber optic cable, where some or all of the reaction components are immobilized on the surface. Therefore, Rothberg et al. do not teach using beads as solid support. Walt et al. teach a system comprising a fiber optic surface with wells into which beads with different functionalities are distributed and which can be used in a variety of biochemical assays, such as sequencing, for example. The advantage of using beads of Walt et al. is that the system provides an enormous flexibility and ease of use, as the fiber optic surface is simply filled with a different set of beads if an assay needs to be changed, saving time and money in building a sensor.

Now let us analyze the alleged "teaching away" statement of Rothberg et al. (col. 21, lines 14-34):

"Solid-phase pyrophosphate sequencing was initially developed by combining a solid-phase technology and a sequencing-by-synthesis technique utilizing bioluminescence (see e.g., Ronaghi, et al., 1996. Real-time DNA sequencing using detection of pyrophosphate release. Anal. Biochem. 242: 84-89). In the solid-phase methodology, an immobilized, primed DNA strand is incubated with DNA polymerase, ATP sulfurylase, and luciferase. By stepwise nucleotide addition with intermediate washing, the event of sequential polymerization can be followed. A remarkable increase in signal-to-noise ratio was obtained by the use of .alpha.-thio dATP in the system. This dATP analog is demonstrated to be efficiently incorporated by DNA polymerase while being silent for luciferase, allowing the sequencing reaction to be performed in real-time. In these early studies, sequencing of a PCR product using streptavidin-coated magnetic

beads as a solid support was presented. However, it was found that the loss of the beads during washing, which was performed between each nucleotide and enzyme addition, was the limiting factor to sequence longer stretches." (emphasis added)

Thus the only conclusion Rothberg et al. draws is that the loss of beads in homogeneous assays (where the beads are suspended in solution) prevented sequencing of "longer stretches", and it is not clear whether "longer stretches" in this case means tens, hundreds or thousands of base pairs. However, Appellant's claims are not drawn to sequencing of any particular lengths of nucleic acids.

Further, let us see what the original reference of Ronaghi et al. (cited in the IDS by Appellant) has to say on the matter of bead loss. On page 87, at the end of third paragraph, Ronagi et al. state:

"The decrease in signal due to loss and aggregation of beads during the washing procedure (measured by the decrease in optical density) has been compensated for in Fig. 5. The loss was lower for the M450 beads (about 2% of the beads were lost per wash) than for the M280."

However, they also offer a solution (page 88, fourth paragraph):

"For instance, by immobilization of the DNA template in a capillary the template loss observed for the paramagnetic beads could be avoided".

Therefore, here it is: immobilize the template to something that cannot flow away with washing solution.

Now, let us look at Walt et al. Is it likely that the beads of Walt et al., distributed in wells on a surface of fiber optic bundle, may be lost? In col. 6, lines 42-67, continued in col. 7, lines 1-10, Walt et al. state:

"Generally in this embodiment, the microspheres are non-covalently associated in the wells, although the wells may additionally be chemically functionalized as is generally described below, cross-linking agents may be used, or a physical barrier may be used, i.e. a film or membrane over the beads.

In a preferred embodiment, the surface of the substrate is modified to contain chemically modified sites, that can be used to attach, either covalently or non-covalently, the microspheres of the invention to the discrete sites or locations on the substrate. "Chemically modified sites" in this context includes, but is not limited to, the addition of a pattern of chemical functional groups including amino groups, carboxy groups, oxo groups and thiol groups, that can be used to covalently attach microspheres, which generally also contain corresponding reactive functional groups; the addition of a pattern of adhesive that can be used to bind the microspheres (either by prior chemical functionalization for the addition of the adhesive or direct addition of the adhesive); the addition of a pattern of charged groups (similar to the chemical functionalities) for the electrostatic attachment of the microspheres, i.e. when the microspheres comprise charged groups opposite to the sites; the addition of a pattern of chemical functional groups that renders the sites differentially hydrophobic or hydrophilic, such that the addition of similarly hydrophobic or hydrophilic microspheres under suitable experimental conditions will result in association of the microspheres to the sites on the basis of hydroaffinity. For example, the use of hydrophobic sites with hydrophobic beads, in an aqueous system, drives the association of the beads preferentially onto the sites. As outlined above, "pattern" in this sense includes the use of a uniform treatment of the surface to allow attachment of the beads at discrete sites, as well as treatment of the surface resulting in discrete sites. As will be appreciated by those in the art, this may be accomplished in a variety of ways."

In col. 18, lines 21-32, Walt et al. state:

"FIGS. 7A and 7B show polymer coated microspheres 12 in wells 250 after their initial placement and then after tapping and exposure to air pulses. FIGS. 7A and 7B illustrate that there is no appreciable loss of microspheres from the wells due to mechanical agitation even without a specific fixing technique. This effect is probably due to electrostatic forces between the microspheres and the optical fibers. These forces tend to bind the microspheres within the wells.

Thus, in most environments, it may be unnecessary to use any chemical or mechanical fixation for the microspheres."

Therefore, Walt et al. provide a whole list of solutions to prevent the beads from flowing away, including covalent immobilization of beads in the wells. They also show that even electrostatic interaction is sufficient to prevent the beads from flowing away from the wells.

In conclusion, faced with the teachings of Rothberg et al. and Walt et al., one of ordinary skill in the art would not be discouraged from using beads of Walt et al., since both Walt et al. and the original paper of Ronaghi et al. present a simple solution to the problem: fix the beads.

Appellant further discusses issues of lateral diffusion of pyrophosphate, which are not relevant to the claims, as no particular spacing between the sites is claimed by Appellant. Appellant then brings up the teaching away of bead loss based on Rothberg's et al. application No. 09/814,338, which is a CIP of the '320 patent, again arguing that Rothberg et al. realized bead loss was a possibility. However, as careful evaluation of the prosecution of that case indicates, bead loss was not an issue on which patentability was based. In fact, the two declarations of Dr. Margulies, one from March 23, 2004 and one from December 28, 2006 were filed in response to claim rejection over the publication of the instant application, US 2003/0108867, over which the claims of Rothberg et al. were rejected. The issue was whether the particular well sizes of Rothberg et al. would be obvious in view of the teachings of the instant application. In the declaration filed April 23, 2004, Dr. Margulies states (page 4, paragraph 15):

"15. As recited in the claims, the compact wafers of the invention include optimally sized fibers and wells that allow maximal signal capture and minimal sample loss and thereby provide

significantly improved sequence analysis. The advantages of the claimed apparatus and substrate are fully disclosed in the instant application, as filed.",

and on page 5 and 6, paragraph 21:

"21. Chee et al. do not appear to specify well depths for use with the optic fibers, and evidently fail to recognize the importance of well depth in preventing sample loss. In fact, Fig. 1 in Chee et al. shows beads and samples jutting out from their wells. This configuration would likely lead to significant sample loss during the "invert and dip" process reported by Chee et al. In contrast, the compact wafer of the invention employs well depths of one-half to three times the diameter of the fiber, which are important in minimizing sample loss during preparation and analysis. The optimally sized fibers and wells (16 and 17, above) therefore represent *significant functional advantages* over the system reported in Chee et al."

In the declaration filed December 28, 2006, Dr. Margulies states (page 2, paragraph 5 and page 3 paragraph 8):

"5. In this patent application, the claims are directed to a substrate comprising a cavitated fiber optic wafer formed from a fused bundle of a plurality of individual optical fibers, each individual fiber having specified dimensions (or to an apparatus having such a substrate). Specifically, each of the claims require that each individual optical fiber has a diameter between 3 and 100 μM, the thickness of the wafer (i.e., length of the optic fiber) between the top surface and the bottom surface is between 0.5 mm and 5.0 mm and the depth of each well ranges from between one half the diameter of an individual optical fiber and three times the diameter of an individual optical fiber. These specific parameters of the claimed cavitated fiber optic wafers are not chosen arbitrarily."

"8. Furthermore, the claimed parameters for well diameter (between 3 and 100gM) and well depth ranging from between one half diameter of an individual optical fiber and three times the diameter of an individual optical fiber are not arbitrarily chosen parameters. Well depth is selected on the basis of a number of competing requirements in a nucleic acid sequencing

application: (1) wells need to be deep enough for DNA-carrying beads to remain in the wells in the presence of convective transport past the wells; (2) the wells must be sufficiently deep to provide adequate isolation against diffusion of by-products from a well in which incorporation is taking place to a well where no incorporation is occurring; (3) they must be shallow enough to allow rapid diffusion of nucleotides into the wells and rapid washing out of remaining nucleotides at the end of each flow cycle to enable high sequencing throughput and reduced reagent use; and (4) they must not be so deep that it would be easy for more than one bead to fit in a well."

It was the combined evidence of the influence of the well depth on the quality of sequencing product, not evidence of a lack of bead loss that persuaded examiner to allow the Rothberg et al. case.

Considering all the emphasis Appellant placed on bead loss during the sequencing process, it seems like a gravely serious issue, and surely Appellant would provide a solution to the problem. However, the only mention of bead loss is provided by Appellant on page 34, lines 34-37, continued on page 35, lines 1-10:

"In a preferred embodiment, when non-covalent methods are used to associate the beads to the array, a novel method of loading the beads onto the array is used. This method comprises exposing the array to a solution of particles (including microspheres and cells) and then applying energy, e.g. agitating or vibrating the mixture. This results in an array comprising more tightly associated particles, as the agitation is done with sufficient energy to cause weakly-associated beads to fall off (or out, in the case of wells). These sites are then available to bind a different bead. In this way, beads that exhibit a high affinity for the sites are selected. Arrays made in this way have two main advantages as compared to a more static loading: first of all, a higher percentage of the sites can be filled easily, and secondly, the arrays thus loaded show a substantial decrease in bead loss during assays. Thus, in a preferred embodiment, these methods are used to generate arrays that have at least about 50% of the sites filled, with at least about 75% being preferred, and at least about 90% being particularly preferred. Similarly, arrays generated

in this manner preferably lose less than about 20% of the beads during an assay, with less than about 10% being preferred and less than about 5% being particularly preferred."

Therefore, Appellant discloses bead loss of at least and up to 20%. In view of the lack of experimental evidence, it is not possible to ascertain whether this values is realistic, i.e., what percentage of beads gets lost during an assay.

In conclusion, the "teaching away" of Rothberg et al. amounts to nothing more than a cautionary note "watch out for possibility of bead loss if they are in solution", to which one of ordinary skill in the art would know a simple solution: fix the beads. The teaching of Walt et al. provides a large number of solutions to potential bead loss. Finally, if the bead loss was such a significant factor in preventing performance of the method, and if the solution to the alleged problem wasn't obvious, Appellant would surely provide a way to avoid bead loss. Instead, Appellant concludes that bead loss up to 20% is expected, and does not conclude that such bead loss would be detrimental to the method.

In view of the above discussion, the rejection of claims 1-16, 22-27, 31-38, 40-42, 44-47, 49 and 50 as unpatentable over Rothberg et al. and Walt et al. is maintained.

Regarding III, in view of the discussion presented above, the rejection of claim 17 as unpatentable over Rothberg et al., Walt et al. and Nyren et al.; the rejection of claims 18, 19, 28-30, 43 and 48 as unpatentable over Rothberg et al., Walt et al., Nyren et al. and Stratagene Catalog; and the rejection of claims 20 and 21 as unpatentable over Rothberg et al., Walt et al., Nyren et al., Stratagene Catalog and Ross et al. are maintained.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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